Attorney Docket No. 59573/46865

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Heegaard, A.M, et al

U.S.S.N.: 10/10,623,150 Group: 1636

FILED: July 18, 2003 Examiner: Garvey, T.L.

FOR: METHOD FOR SCREENING COMPOUNDS FOR ACTIVITY IN TREATING AN OSTEOCLAST RELATED BONE DISEASE

Mail Stop: Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. §1.132

Dear Sir:

I, Morten Karsdal, declare I am an inventor of claims in the above-identified application (hereinafter "Application").

I obtained the Master of Science degree at "the Technical University of Denmark" in 1998, and a PhD degree from "the Southern University of Denmark" in 2005. Presently I am the Head of Pharmacology at Nordic Bioscience. For more than 8 years I have been working in the field of pharmacology of bone and cartilage, including screening of compounds against ClC-7. I have more than 30 Pubmed citations within the field of bone/cartilage and acidification of endosomal compartments including the function of ClC-7.

I have recently read the Application and the Office Action dated September 11th, 2006 ("Office Action") issued in the Application. I wish to provide the following comments for the Examiner's consideration.

The Examiner has stated (page 5) that *'the applicant has not demonstrated that the screening method works to identify even one compound with potential activity to treat any osteoclast related disorder. Further, the compound screening assay is conducted in the human embryonic kidney cell line HEK293 that recombinantly express ClC-7 and are not related to an osteoclast. Additionally, the applicants have not demonstrated that any osteoclast disorder will be affected by blocking ClC-7.'* The Examiner further stated *'Schaller et al demonstrates analysis of the chloride channel inhibitor NS3736 for its ability to inhibit chloride channel conductance in differentiated osteoclasts and its ability to inhibit bone resorption in vitro and in the OVX rat model. Schaller further determines which chloride channel may be inhibited by the NS3637 compound by expression pattern analysis of the ClC-7 and CLIC1. The expression of ClC-7 on osteoclasts suggests that NS3736 may inhibit ClC-7. Schaller et al does not teach identifying compounds for the treatment of osteoporosis by screening compounds specifically for their ability to block ClC-7 and do not directly demonstrate that NS3736 blocks ClC-7'.*

In the experiments described below, we have demonstrated that inhibition of acidification by NS3736 occurs in HEK 293 cells overexpressing ClC-7. Consequently, the screening method does work to identify compounds with potential activity to treat any osteoclast related disorder.

Acidification Inhibition by NS3736

Acridine orange dye was used to investigate the response of the kidney cells HEK 293 cells overexpressing ClC-7 to NS3736. Acridine orange emits a bright orange fluorescence at a pH between 4 and 5 due to acidification. Without acidification, acridine orange fluoresces green. To perform the acidification assay, the HEK 293 cells overexpressing ClC-7 was used, and the cells were seeded in 96 well plates (50,000 cells/well) and cultured for 1 day. Acridine orange (3,6-bis[Dimethylamine]acridine) at 10 μg/ml was loaded for 45 min in the culture medium of HEK cells in the presence or absence of bafilomycin or NS3736 as inhibitors. The dye was washed and pictures were taken using an OLYMPUS IX-70 microscope and an OLYMPUS U-MWB filter (20X objective) or the fluorescence were measured using the SpectraMax Gemini

XS at excitation 492 nm and emission 535 nm. The acidification was also measured quantitatively by measurement of the fluorescence of the green signal (inhibited acidification). This was done in the SpectraMax Gemini XS done at excitation 492 nm and emission 535 nm (green wavelength).

As seen in figure A attached, HEK cells in the absence of inhibitors have an orange fluorescence, while addition of NS3736 led to complete abrogation of the orange signal at 50 μM. NS3736 is seen to inhibit the acidification dose dependently. The known V-ATPase inhibitor, bafilomycin A1 (200 nM), was used as a positive control showing the same as NS3736, as expected[1,2]. As seen in figure B attached, NS3736 inhibits the acidification dose dependently resulting in a higher fluorescence of the green wavelength. As seen in Figures A and B, Bafilomycin, as a positive control, inhibits the acidification totally at 200 nM. In panel A, NS3736 inhibits acidification dose dependently and totally at 50 μM. In panel B, NS3736 inhibits the acidification dose dependently.

These results demonstrate that the screening assay described and claimed in the application would succeed in identifying NS3736 as a potential inhibitor of ClC-7 mediated acidification. Schaller et al demonstrates that NS3736 is a potent inhibitor of osteoclast mediated bone resorption both *in vitro* and in the OVX rat model *in vivo*. In combination, these results verify that practice of the invention can successfully identify compounds effective to inhibit bone resorption by osteoclasts which are therefore good candidates for the treatment of osteoclast related disorders including osteoporosis and related conditions.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

9/1-2007
Date

Morten Karsdal

Reference List

1. Karsdal,M.A., Henriksen,K., Sorensen,M.G., Gram,J., Schaller,S., Dziegiel,M.H., Heegaard,A.M., Christophersen,P., Martin,T.J., Christiansen,C. & Bollerslev,J. Acidification of the osteoclastic resorption compartment provides insight into the coupling of bone formation to bone resorption. *Am J Pathol* **166**, 467-476 (2005).

2. Yoshimori,T., Yamamoto,A., Moriyama,Y., Futai,M. & Tashiro,Y. Bafilomycin A1, a specific inhibitor of vacuolar-type H(+)-ATPase, inhibits acidification and protein degradation in lysosomes of cultured cells. *J Biol Chem* **266**, 17707-17712 (1991).